RECEIVED
CENTRAL FAX CENTER
NOV 0 9 2006

REMARKS

Regarding the Amendments

Claim 1 has been amended as set forth in the above Complete Listing of the Claims.

As amended, the claims are supported by the specification and the original claims. No new matter (35 U.S.C. § 132) has been added. As amended, the claims are supported by the specification and the original claims.

The amendments do not require a new search, or raise new issues for consideration because they merely address issues already raised by the Examiner and define Applicants' invention more clearly.

It is submitted that the amendments place the claims in condition for allowance or in better condition for appeal by reducing the number of issues for consideration on appeal.

The amendments were not made earlier in the prosecution because it is maintained that the previously pending claims were allowable. Since the amendments do not add new matter or require a new search or consideration, and place the claims in conditions for allowance or in better condition for appeal, entry of the amendment is respectfully requested.

Upon entry of the amendments, claims 1-14 and 21-24 will be pending.

Submission of Replacement Drawings for Figure 3B

Enclosed in Appendix A hereof is a new Figure 3B (5 pages in total). Consistent with the requirements of 37 CFR 1.121(d), each of the replacement sheets of formal drawings has been labeled in the header thereof as "REPLACEMENT SHEET."

The replacement Figure 3B is the same as originally submitted with the application, but has been rendered with greater clarity and slightly larger font. The numbering of the sequence throughout the whole of Figure 3B is now consistent from Figure 3B(I) to 3B(V), top to bottom of each page. It is respectfully submitted that these replacement drawings are acceptable corrected drawings, as requested in the Office Action mailed August 9, 2006, and their entry is respectfully requested.

Withdrawal of the drawing objection is therefore respectfully requested.

Rejection of Claims 1-14 and 21-24 Under 35 U.S.C. §112, first paragraph

In the August 9, 2006 Office Action, the specification was objected to and claims 1-14 and 21-24 were rejected under 35 U.S.C. § 112, first paragraph as lacking sufficient written description and enablement. Applicant respectfully traverses the rejection.

The balance of the Office's position rests on the reiterated assertion that separation of the secreting and non-secreting cells is difficult from a population of cells that may all bind the secreted product.

Applicant respectfully submits that the selection of antibody expressing cells is described in the present application and is enabled by the present application, as set forth below.

The Examiner's attention is respectfully drawn to claim 1, as amended. Claim 1 has been amended in order to recite Applicant's invention more clearly, in accordance with the statement on page 1 of the application "that monoclonal antibodies on the cell surface of hybridoma cells can be presented by means of an antibody binding protein." Claim 1 recites a method of preparing and selecting monoclonal antibodies based on selection of those hybridomas that present an antibody on the surface. Expression of the antibody binding protein on the surface of myeloma cells and on generated hybridoma cells and detection of a presented antibody bound thereto have been clearly set forth in the application.

A large amount of antibody binding protein is stably expressed on the surface of myeloma cell line X63-Ag8.653.3. Indeed, without any selective pressure, e.g., G418 selection, this cell line exhibited stable and very strong expression of surface displayed protein G for months. These statements are addressed in the Office Action mailed August 9, 2006 on page 5 and are not contested by the Office.

Applicant agrees with the statement on page 3 of the Office Action mailed August 9, 2006 that "retention of the antibody binding protein in the hybrid cells" cannot be assumed for every descendent of the hybridoma cells generated by cell fusion. Indeed, congruent with this notion, the FACS data provided in Applicant's response filed July 17, 2006 clearly shows that 14-days-post-fusion most individual hybridoma cells generated by the fusion with protein G-expressing myeloma cell line X63-Ag8.653.3 do not give a signal when stained with FITC-labeled goat-anti-

mouse antibodies. Applicant respectfully submits that the claims do not recite retention of the antibody binding protein in each and every individual descendent cell, but instead recite a method to separate descendents that display antibodies from those that do not.

In order to separate antibody-displaying descendents from those that do not, as clearly shown by the FACS data provided in Applicant's July 17, 2006 response:

1. Post-fusion of some antibody-producing hybridoma cells should retain the antibody binding protein (and the antibody gene);
2. the frequency of those cells should be high enough to be within reach, *e.g.* of FACS sorting machinery; and
3. the signal due to surface displayed antibodies should allow for the discrimination of antibody-presenters from non-presenters.

The FACS data clearly shows that for nearly every clone of hybridoma cells, even 14 days post fusion, at least a minority of cells gives a clear signal up to the third decade of surface displayed mouse antibodies, and this signal is definitively strong enough to discriminate mouse-antibody presenters from non-presenters.

In the response filed July 17, 2006, Applicant asserted that cell selection by FACS is within the skill of those in the art. On page 4 of the Office Action mailed August 9, 2006, it is stated that "[t]he issue is not if one can select cells by FACS..." It is Applicant's position that these cells can be selected by FACS, that the signal would be strong enough to FACS select those cells that do display mouse antibodies from those that don't. Thereby, the second part of the statement on page 4 of the Office Action mailed August 9, 2006 remains at the center of the dispute "...whether the selected cells are the secreting cells..."

The Examiner's attention again is respectfully drawn to claim 1, as amended to particularly specify in a clear and distinguishing manner the method of the invention. Applicants' claimed method is directed to detection of hybridomas presenting antibodies bound to the antibody binding proteins on the surface thereof. It is well described in the application and in material previously presented to the Office that there is a high affinity between the expressed antibody binding protein and the monoclonal antibodies that are ultimately detected. In particular, it was acknowledged in the Office Action mailed August 9, 2006 on page 5 that goat antibodies (it is assumed here that cow antibodies from FCS were intended) bind to protein G with high affinity.

As previously presented, individual cells from nearly every hybridoma clone generated with a protein G expressing myeloma cell line clearly stain with FITC-labeled-goat-anti-mouse-

antibodies. Please note that the vast majority of hybridoma clones exhibit at least some cells with a frequency >0.1% that stain brightly with FITC-labeled-anti-mouse-antibodies, up to the third decade. In order to investigate the influence of cross-talking (cow-) antibodies, these experiments were done under extremely unfavorable conditions for preservation of or competitive binding of endogenously produced mouse-antibodies.

Specifically, post-fusion endogenously produced mouse antibodies and competing cow antibodies from FCS were co-incubated over a lengthy period of 14 days, which is enough time for competing cow-antibodies to displace protein G bound mouse-antibodies. Experiments were done with repeated washings over the time period in order to remove the majority of secreted mouse-antibodies (and at the same time replenish 10% FCS), thus resulting into a huge surplus (>1,000 fold) of protein G-binding cow-antibodies (from repeatedly added 10% FCS) over endogenously produced mouse-antibodies. Previously presented FACS data clearly shows that even under these conditions quite a few individual hybridoma cells do display mouse-antibodies and others do not. Therefore, conditions that forcefully and artificially promote the uptake of high-affinity protein G-binding "cross-talking" cow-antibodies from the medium by surface-displayed protein G still yield a minority of cells that display mouse-antibodies with a frequency that is similar to the frequency of positive, *i.e.* still-antibody-producing hybridoma cells expected from normal sub-cloning experiments.

The majority of interactions are therefore uptake of cross-talking cow-antibodies by protein G displaying hybridoma cells. However, this results in a minority of individual cells that still display mouse antibodies on their surface (as seen by bright staining of some individual cells up to the third decade). The experimental conditions described above were chosen to rule out a significant influence of cross talking mouse-antibodies. Such mouse-antibodies are removed by consecutive washings and are minimally present when compared with the overwhelming presence of cow-antibodies. Therefore, in theory, if an equitable comparison of mouse- and cow-antibodies had been done, none of the hybridoma cells should have displayed a measurable amount of mouse antibodies. However, a minority of cells clearly does display a measurable amount of mouse antibodies, even though competition of mouse- vs. cow-antibodies was allowed for a time period of 14 days. Therefore, these cells must display their endogenously produced individual antibody simply because they couldn't get a cow antibody from elsewhere.

It is also well known in the art that antibodies and antibody binding proteins travel the same export pathway down the ER. While traveling this pathway, the mouse antibodies are pre-bound

by the large amount of protein G. Thus, each hybridoma's surface is constantly refueled with its cell-specific antibody, provided the hybridoma cell produces protein G together with an antibody. Applicant previously provided citations where a similar antibody-antigen binding within the ER was described.

Enclosed in Appendix B hereof is an additional citation that describes the commercially available Zenon labeling kit from molecular probes (as available online at hyper text transfer protocol (http) address probes.invitrogen.com/media/pis/mp25000.pdf), to support Applicant's claim that the kinetic advantage of pre-bound antibodies over competing antibodies added later is a well-known phenomenon. With these kits monoclonal mouse antibodies are reacted for 5 minutes with labeled secondary antibodies, while surplus secondary antibodies are simply removed by binding to excess "unspecific" polyclonal mouse antibodies.

These Zenon Kits not only allow for specific labeling due to a kinetic advantage, but also to pool several differently labeled monoclonal mouse antibodies, and thus, *e.g.*, FACS-label one cell with two or three different monoclonal antibodies in one reaction. This simple procedure works although each individual and differently labeled secondary antibody is directed against the very same constant regions displayed on mouse monoclonal antibodies.

It is noteworthy that the antibody concentration within the ER of an antibody producing hybridoma cell exceeds the Zenon-Kit-recommended concentrations. Therefore, the affinity of protein G towards mouse-IgG is similar to a high-affinity antibody-antigen reaction, and not all labeled secondary antibodies need to be trapped in the first instance to generate specifically labeled monoclonal antibodies by the Zenon Kit.

On page 4 of the Office Action mailed August 9, 2006, it is contended that Applicant asserts pre-binding or "sufficient quantities of binding protein to effectively intracellularly bind and trap all antibodies produced." (Emphasis added.) Alternatively, the Office states that "applicant's specification provides no written description or guidance for inhibiting the uptake of secreted antibodies by undesired non-secreting cells in the population, if the cells have the instantly disclosed antibody binding protein expressed thereon" (Office Action, page 3). Applicant respectfully submits that the claims do not recite or require binding and trapping all antibodies produced. It is further apparent that the claim requires "expressed antibodies... presented as bound to antibody binding proteins on the surface of the hybridoma cell" and "detecting ... hybridoma cells containing presented antibodies by a labeled specific antigen that binds to the

presented antibody" for subsequent selection of a hybridoma cell presenting the monoclonal antibody, and that therefore the invention requires only the binding of **enough** antibodies to be recognized or detected, *e.g.* in FACS analysis.

Applicant further points out that relevant protein concentrations within the ER, affinities of reactants, and the time needed to travel down the ER are even more favorable for a kinetic advantage when compared to the commercially available Zenon Kit.

Applicant also points to the FACS data previously presented, where a minority of hybridoma cells shows a strong signal of surface displayed antibodies even under the most unfavorable conditions. In addition, different Zenon Kit labeled monoclonal antibodies can be mixed, and still differentiate their different antigens. This is in contrast to the Office's assertion that such differentiation could not be performed, due to cross-talking antibodies.

Based on the foregoing, it would have been clear to one of skill in the art at the time of filing of the present application, how to select hybridoma cells presenting the desired monoclonal antibody and not cells which fail to present the antibody.

Accordingly, the invention of claims 1-14 and 21-24 is sufficiently described and is enabled by the present specification.

Applicant maintains that the present application fully sets forth the specific starting material and the conditions under which the claimed method could have been carried out at the time of filing of the invention, without undue experimentation. The only specific starting material needed to generate hybridoma cells with surface displayed antibodies for use in the claimed methodology of applicants' invention is a myeloma cell line that is engineered to display antibody binding proteins, *e.g.* protein G linked to the membrane.

Due to the kinetic advantage of protein G-pre-bound antibodies in the ER, some hybridoma cells display a strong signal of surface displayed endogenously produced antibody over a majority of unlabeled cells. This is evidenced by hybridoma cells brightly stained with anti-mouse-antibodies even under the most unfavorable experimental conditions. The myeloma cell line, and the DNA used to generate it, are set forth in the specification of the present application. The specification shows a stable display of large amounts of protein G over months, and further tested in Applicant's laboratory to yield exactly the same level as of hybridomas when compared to the parental cell line X63-Ag8.653 (158 hybridomas per mouse vs. 155 hybridomas).

On page 5 of the August 9, 2006 Office Action, it was noted that the pending claims are not limited to a myeloma cell line that exhibits a stable display of large amounts of protein G and in particular that more than X63-Ag8.653.3 myeloma cell line is claimed. In response, applicants respectfully submit the following:

1. It is clear that the particular myeloma cell line X63 exhibits a stable display of large amounts of protein G. Based on the instructions given in the specification of the application, *e.g.* with regard to vectors, one of skill would have been able to construct further myeloma cell lines based on these instructions at the time of filing of the present application, as encompassed by the method of claim 1. It is apparent in light of the specification that the claimed methods utilize myeloma cells that exhibit a stable display of substantial amounts of protein G.

2. There are many examples of proteins, *e.g.* fluorescence proteins like EGFP or dsRED (see *e.g.* world wide web address clontech.com) which are well-expressed in completely different cell lines that have absolutely nothing to do with the source from which the proteins were derived.

3. As is well known, for expression of a gene obtained from bacteria, the codons of the gene have to be adapted for expression in eukaryotic cells. This is, however, disclosed in detail in the present application and the deposited plasmids show the person skilled in the art exactly how to modify the codons for expression in eukaryotic cells.

4. In addition, the Examiner's attention is respectfully drawn to page 1, first paragraph of the application, disclosing preferred expression vectors, which contain a DNA according to the present invention and which can be used for transfecting any myeloma cell for stable expression of large amounts of an antibody binding protein like protein G.

5. Myeloma cells are restricted to cell lines of vertebrates since only vertebrates possess B-cells/antibodies. Moreover, these cells show a high degree of differentiation and specialization since all myeloma cells are derived from antibody producing plasma cells. In other words, various kinds of myeloma cell lines show a high degree of similarity, which is an additional reason why one of skill in the art would have used results obtained by the particular cell line X63-Ag8.653.3 and would have been able to extrapolate to other myeloma cell lines.

6. In addition to the cell line X63-Ag8.653.3, the present application shows additional cell lines expressing the antibody binding protein, *e.g.* the hybridoma cell line U98/6; see Example 2 of the present application. This example shows that the hybridoma cells have a green fluorescence. This fluorescence is due to the expression of antibodies on the cell surface of hybridoma cells. Moreover, the different hybridoma cell lines derived from the fusion with the protein G expressing myeloma sub-cell line of X63-Ag8653.3 that were used for the FACS analyses attached to our petition of July 17, 2006 show that the antibody binding protein G was already successfully expressed in a variety of different B-cells.

7. The present application discloses at least three different antibody binding proteins (Protein G, Protein L and Protein LG) expressing vectors and these antibody binding proteins worked in different cell lines related to B-lymphocytes. This shows that the present application is not only enabling for the stable expression of large amounts of protein G but also for expression of additional antibody binding proteins. Finally, the specification lists further antibody proteins such as protein A, CD16, CD32 and CD64 (see specification, page 4, 1st full paragraph).

Therefore, in light of the above discussion, it is clear that the present specification provides adequate written description and enablement for the claimed methods of claims 1-14 and 21-24.

CONCLUSION

Based on the foregoing, all of Applicant's pending claims 1-14 and 21-24 are patentably distinguished over the art, and are in form and condition for allowance. The Examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

No fees are believed to be due for the filing of this paper. However, should any fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the Examiner is requested to contact the undersigned attorney at (919) 419-9350 to discuss same.

Respectfully submitted,

Steven J. Hultquist
Reg. No. 28,021
Attorney for Applicants

INTELLECTUAL PROPERTY/
TECHNOLOGY LAW
Phone: (919) 419-9350
Fax: (919) 419-9354
Attorney File No.: 4121-126

Enclosures:
Appendix A - Revised Figure 3B
Appendix B – Zenon® Mouse IgG Labeling Kits

The USPTO is hereby authorized to charge any deficiency or credit any overpayment of fees properly payable for this document to Deposit Account No. 08-3284

APPENDIX A